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Chromosoma Focus

Review:

Assigning functions to nucleolar structures

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Nucleoli provide the fascinating possibility of linking morphologically distinct structures such as those seen in the electron microscope with biochemical features of the formation and stepwise maturation of ribosomes. Localization of proteins by immunocytochemistry and of rRNA genes and their transcripts by in situ hybridization has greatly improved our understanding of the structural-functional relationships of the nucleolus. The present review describes some recent results obtained by electron microscopic in situ hybridization and argues that this approach has the potential to correlate each step of the complex pre-rRNA maturation pathway with nucleolar structures. Evidence is accumulating that the nucleolus-specific U3 snRNPs (small nuclear ribonucleoprotein particles) participate in rRNA processing events, similar to the role played by the nucleoplasmic snRNPs in mRNA maturation. The intranucleolar distribution of U3 snRNA is consistent with the view that it is involved in both early and late stages of pre-rRNA processing.

The nucleolus: a paradigm for nuclear order

The genes coding for ribosomal RNA (rRNA) are unique in that their activity leads to the formation of a recognizable nuclear structure, the nucleolus. Nucleoli are the sites of ribosome biogenesis. They provide the structural framework upon which the synthesis, processing and transport of the rRNA precursor molecules (pre-rRNA), as well as their association with ribosomal and nonribosomal proteins, are coordinated in a time- and space-dependent manner. Thus, the nucleolus might be envisaged as an assembly line linking transcriptional and post-transcriptional events and providing the specific topological order required for the concerted interactions of the multiple components involved in ribosome production. In fact, several lines of evidence, in particular simultaneous autoradiographic localization and biochemical analysis of radioactively labelled RNA, have clearly established that ribosome formation is a vectorial process that can be correlated with morphologically distinct nucleolar components (Das et al. 1970; reviewed in Fakan and Puvion 1980; Puvion and Moyne 1981; Goessens 1984).

Three nucleolar elements are usually identified by thin-section electron microscopy (Fig. 1): the *fibrillar center(s)*, a closely apposed layer of tightly packed fibrils termed *dense fibrillar component*, and the *granular component* constituting the bulk of the nucleolus in metabolically active cells. Ribosome formation begins within the nucleolus with the synthesis of the primary rRNA precursor comprising the mature 18S, 5.8S and 28S rRNA sequences, as well as non-conserved sequences (the so-called transcribed spacers; see Fig. 2) and proceeds by a well-established cascade of endo- and

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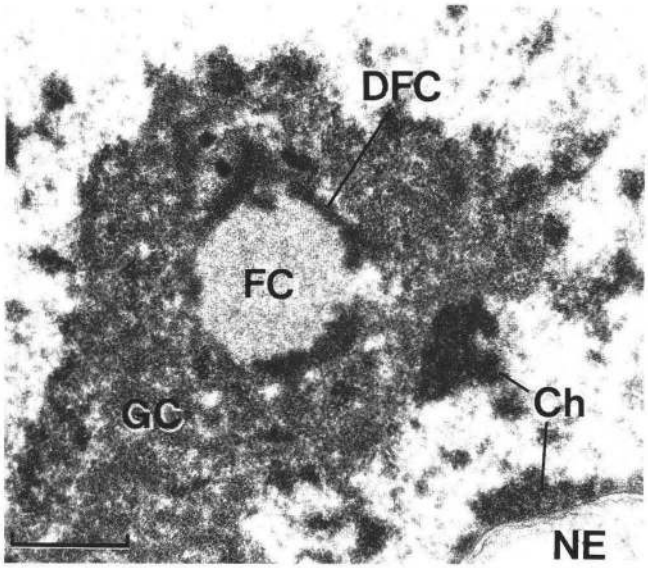


Fig. 1. Electron micrograph showing the nucleolus of a cultured mouse fibroblast with its three major components: *FC*, fibrillar center; *DFC*, dense fibrillar component; *GC*, granular component. Condensed chromatin (*Ch*) is associated with the nucleolar periphery and the nuclear envelope (*NE*). Bar represents 0.5 μm

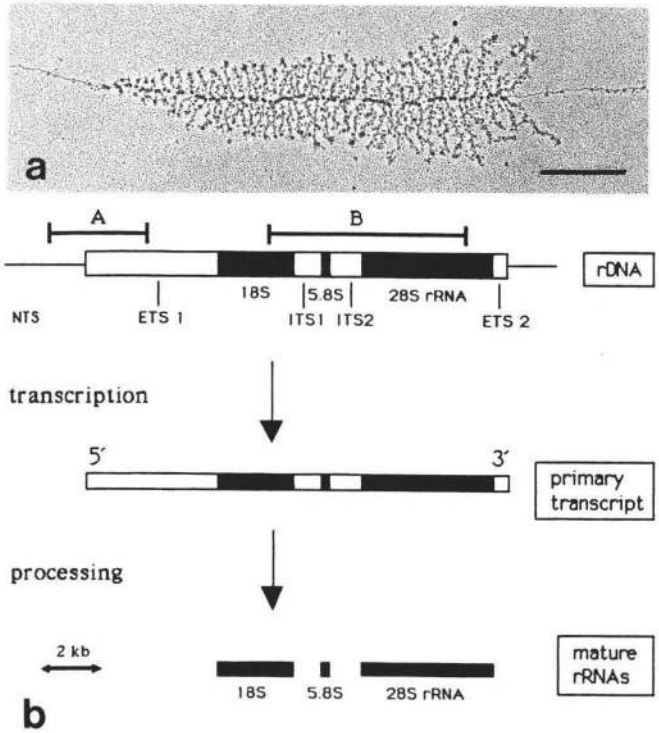


Fig. 2a, b. A transcribing rRNA gene with adjacent nontranscribed spacer regions as seen in an electron microscopic chromatin spread preparation (**a**). Transcription proceeds from left to right. Each lateral fibril is anchored to the chromatin axis by a RNA polymerase I molecule, visible as a dark granule, and contains a nascent pre-rRNA chain with associated proteins. Note the terminal thickening of the free ends of the transcript fibrils. Bar represents 0.5 μm. Below (**b**) is a map of the approximately 14 kb mouse rDNA transcribed region (*boxed*; for details see Sollner-Webb and Mougey 1991) with the surrounding nontranscribed spacers (*NTS*). Regions corresponding to the mature rRNAs are black and transcribed spacer regions (*ETS*, external transcribed spacer; *ITS*, internal transcribed spacer) are white. The primary transcript is processed into the mature 18S, 5.8S and 28S rRNAs by a series of endo- and exonucleolytic cleavages in the transcribed spacer regions. The two rDNA hybridization probes described in the text are marked *A* and *B*

exonucleolytic cleavages, methylation events and assembly with specific nucleolar proteins (for details, see Hadjiolov 1985; Sommerville 1986; Gerbi et al. 1990; Reeder 1990; Warner 1990; Sollner-Webb and Mougey 1991). There is no doubt that the structural organization of the nucleolus reflects in some way this maturation process even though the exact structure-function relationships are as yet largely unclear.

Nucleoli reform after mitosis by the coalescence of discrete structural entities, the so-called prenucleolar bodies, around the chromosomal nucleolus organizing regions (NORs) containing the tandemly repeated rRNA genes. Thus, a nucleolus usually harbors a cluster of rRNA genes. Recent experiments, however, have indicated that only a single rRNA gene is sufficient to organize a nucleolus. Upon P-element mediated insertion of individual rDNA repeat units into a variety of chromosomal sites in *Drosophila*, additional nucleoli were generated resembling their endogenous counterparts (Karpen et al. 1988). Other experiments based on microinjection of antibodies to RNA polymerase I into mitotic mammalian cells have established that transcriptional activity of the rRNA genes is essential for postmitotic nucleolar assembly (Benavente et al. 1987). We suppose that the nascent transcripts rather than the chromatin itself act as nucleation sites for the ordered assembly of the nucleolus since their free ends containing the 5′-regions of the growing pre-rRNA chains are capable of interacting with fibrillarin, a protein component of the prenucleolar bodies and the dense fibrillar component of interphase nucleoli (Scheer and Benavente 1990).

Molecular architecture of the nucleolus

A large number of electron microscopic cytological approaches has been employed to pinpoint the exact intranucleolar location of the transcriptionally active rRNA genes. These include autoradiography of cells pulse-labelled with tritiated uridine, selective DNA staining, in situ hybridization with appropriate rDNA probes, immunolocalization of intranucleolar DNA by using antibodies directed against DNA, and immunogold detection of enzymes involved in rDNA transcription such as RNA polymerase I and DNA topoisomerase I (summarized in Scheer and Benavente 1990; Derenzini et al. 1990; Thiry et al. 1991). These studies have clearly shown that the fibrillar centers are the sole nucleolar elements where rDNA as well as the transcriptional machinery coexist. Although no consensus among investigators has yet been achieved (see, e.g., Jordan 1991;

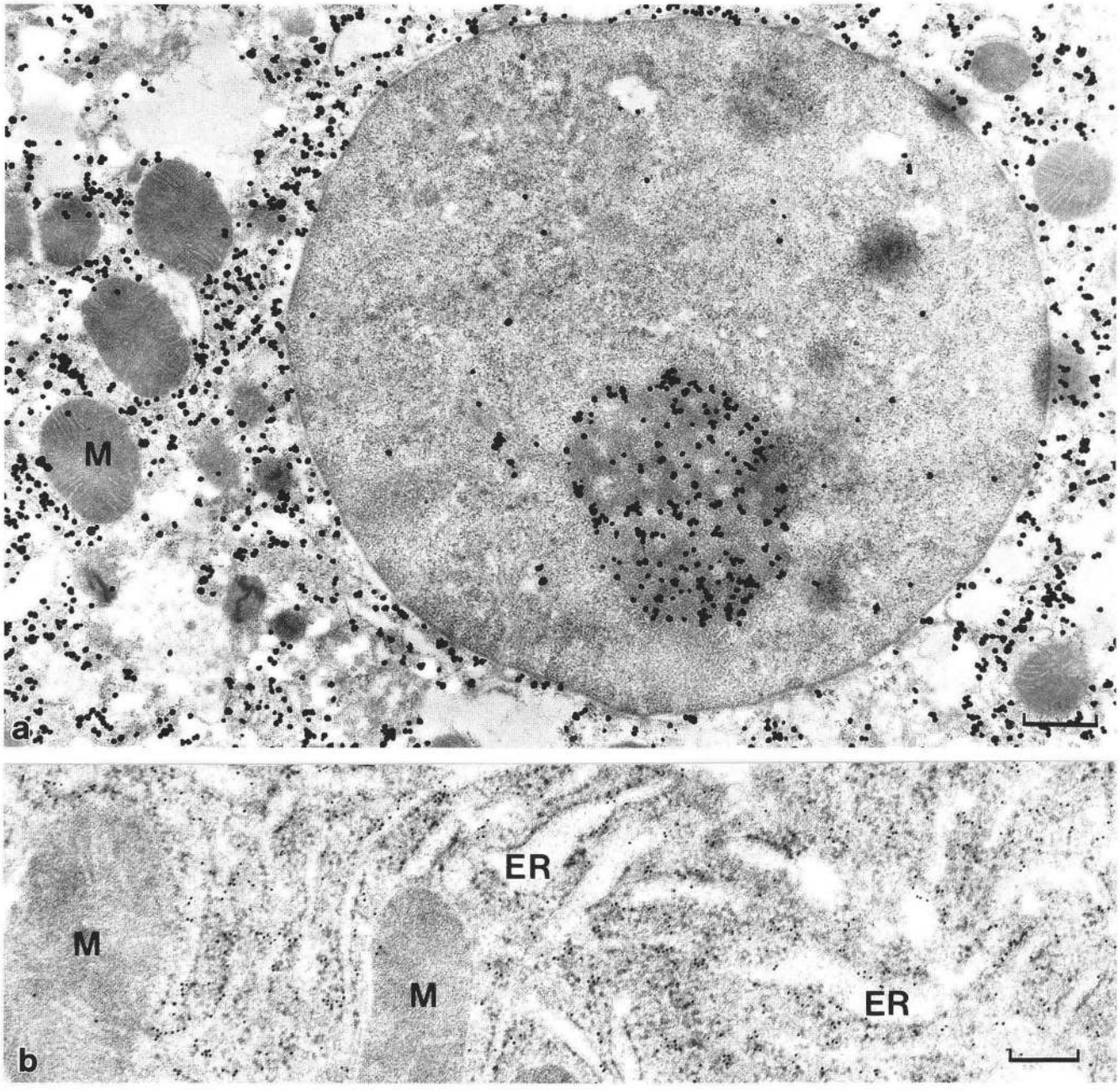


Fig. 3a, b. In situ hybridization of a RNA probe complementary to most of the 28S rRNA region (probe B in Fig. 2) to an ultrathin section of mouse liver, fixed with 3% formaldehyde and embedded in Lowicryl. The "anti-sense" RNA was synthesized in vitro in the presence of digoxigenin-UTP (Boehringer, Mannheim). The hybridized probe was detected either with monoclonal anti-digoxigenin antibodies coupled to 1 nm gold particles (Boehringer, Mannheim) followed by silver enhancement with Aurion R-Gent (Biotrend, Cologne, FRG); **a** or with unconjugated antibodies followed by secondary antibodies coupled to 10 nm gold particles (Aurion); **b** The method of silver intensification facilitates visualization of the bound hybridization probe at low magnification (**a**). The nucleolus as well as the cytoplasm are clearly marked. Label is essentially absent from the mitochondria (*M*), demonstrating the specificity of the method. The few silver grains scattered throughout the nucleoplasm might indicate transport of preribosomal particles from the nucleolus to the cytoplasm. Higher magnification and probe detection with 10 nm gold-antibody complexes reveals that the cytoplasmic labelling is due to labelling of the ribosomes, both free and endoplasmic reticulum (*ER*)-associated ribosomes (**b**). Bars represent 0.5 μm (**a**) and 0.2 μm (**b**)

Hernandez-Verdun 1991), we think it is reasonable to conclude that transcription of the rRNA genes occurs in the fibrillar centers.

Little is known how nucleolar structures and the various stages of the formation of the ribosome are linked. Ideally, one would like to locate in situ the successive maturation steps of the rRNA precursor molecules and to identify the proteins binding to the nascent preribosomes during their flow through the nucleolus. For one ribosomal (S1; Hügle et al. 1985a) and two non-ribo-

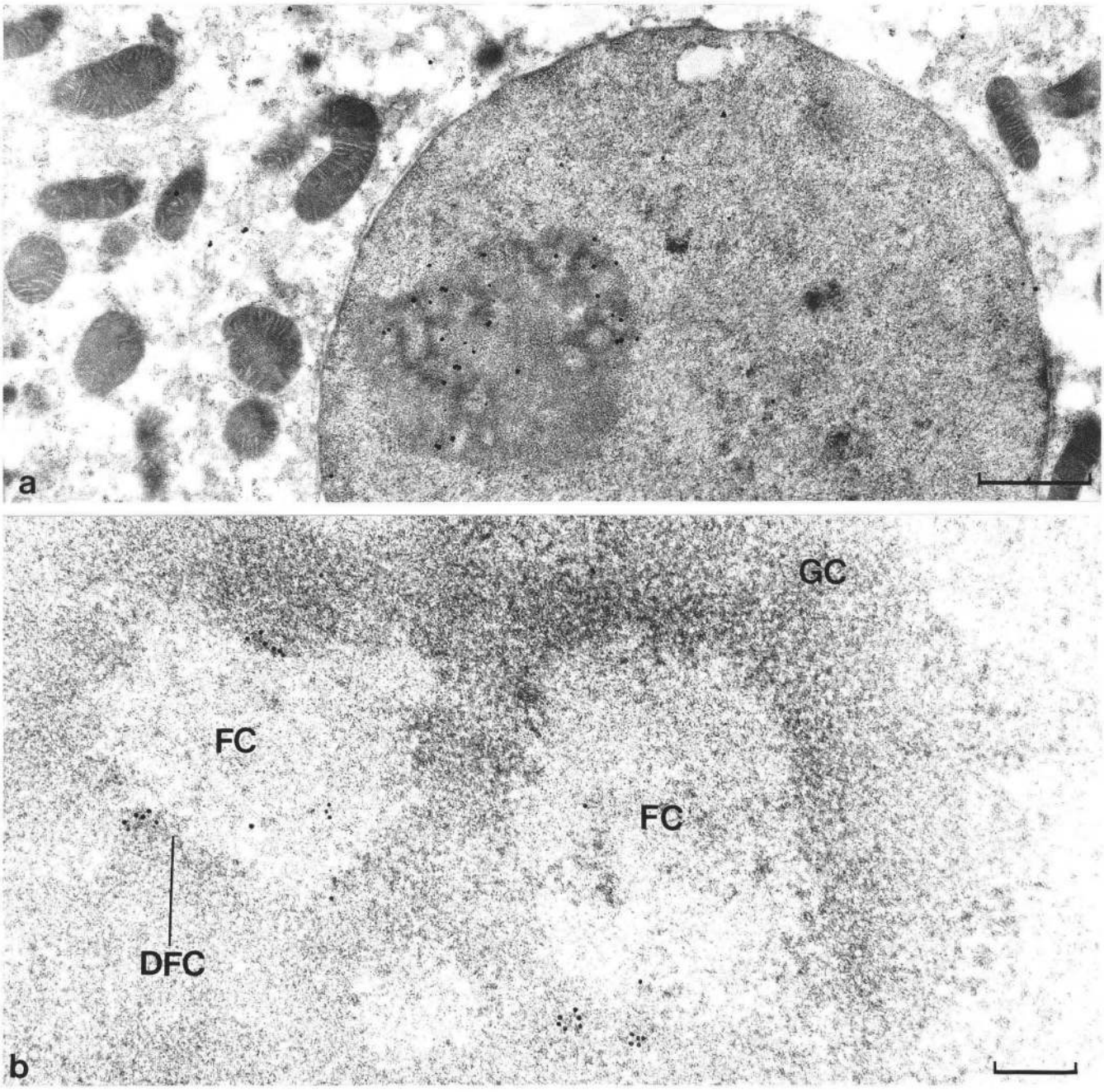


Fig. 4a, b. Localization of pre-rRNA molecules containing the 5′ region of the ETS1 region. A digoxigenin-labelled riboprobe complementary to the first 3 kb of the 5′ region of the ETS1 (probe A in Fig. 2) was hybridized to a Lowicryl section of mouse liver and detected either with monoclonal anti-digoxigenin antibodies coupled to 1 nm gold particles (Boehringer, Mannheim) and silver enhancement (**a**) or with anti-digoxigenin antibodies, followed by secondary antibodies coupled to 10 nm gold particles (**b**). The survey view shows selective labelling of the fibrillar components of the nucleolus (**a**). At higher magnification (**b**) gold particles are seen over the fibrillar centers (*FC*) and a thin layer encircling the fibrillar centers, i.e., the dense fibrillar component (*DFC*). The granular component (*GC*) is essentially free of label. Bars represent 1 μm (**a**) and 0.2 μm (**b**)

somal proteins (C23 or nucleolin and B23 or NO38; Schmidt-Zachmann et al. 1987; Biggiogera et al. 1989) the nucleolar sites of association with preribosomes have been determined. An M_r 40000 protein specifically associated with preribosomal particles of the granular component, originally termed ribocharin (Hügle et al. 1985b), turned out to be an isoelectric variant of B23.

The other aspect of preribosome maturation concerns the multistep processing of precursor molecules to the mature rRNAs of functional ribosomes, which occurs mainly in the nucleolus. At the molecular level these processing events are understood in considerable detail and some of them can already be studied in cell-free systems (see Warner 1990; Sollner-Webb and Mougey

1991). Non-radioactive in situ hybridization methods recently developed now allow assignment of defined processing steps to distinct nucleolar substructures. Progress in this direction will be discussed and a few examples presented.

Spatial order of pre-rRNA processing

In a pioneering study, Thiry and Thiry-Blaise (1989) showed that hybridization of a biotinylated mouse rDNA probe encompassing most of the 18S rRNA sequences to ultrathin sections of mouse cells allowed specific detection of the RNA molecules of cytoplasmic ribosomes and nucleoli. These authors have used Ehrlich ascites cells embedded in Lowicryl (a hydrophilic resin in order to ensure accessibility of the RNA targets) and immunocytochemical identification of the hybridized probe with biotin-antibodies followed by secondary antibodies coupled to gold particles. Since the probe they have used hybridized to conserved (18S) sequences of the pre-rRNA, essentially all nucleolar components were labelled. Actual flow of the RNA molecules from one nucleolar compartment to the next compartment was visualized in a subsequent study in which additional probes specific for transcribed spacer sequences, i.e., sequences that are eliminated during processing, were used to locate specific pre-rRNAs (Puvion-Dutilleul et al. 1991).

An example illustrating hybridization of an in vitro transcribed "anti-sense" RNA probe complementary to most of the 28S rRNA region (probe B in Fig. 2) to an ultrathin section of Lowicryl-embedded mouse liver is presented in Fig. 3a (the corresponding "sense" probe provides a convenient control to monitor specificity of labelling). The nucleolus is clearly marked as well as the cytoplasm (Fig. 3a). At higher magnification, the cytoplasmic labelling can be related to ribosomes, in particular to those of the rough ER (Fig. 3b; in order to achieve high resolution, the anti-digoxigenin antibodies were revealed in this case with secondary antibodies coupled to 10 nm gold particles without silver enhancement). The weak and disperse labelling of the nucleoplasm (Fig. 3a) most likely reflects the occurrence of transport forms of preribosomal particles.

In contrast, riboprobes complementary to the 5′ external transcribed spacer (ETS 1; probe A in Fig. 2) hybridize almost exclusively to the fibrillar centers (with some preference for their peripheral regions) and the dense fibrillar component (Fig. 4a, b). This intranucleolar distribution indicates that after their release from the rDNA template, the transcripts migrate from the fibrillar center into the surrounding dense fibrillar component (see also Puvion-Dutilleul et al. 1991). Here removal of ETS 1 from the pre-rRNA, which is a relatively early event in the maturation pathway, occurs before the preribosomes pass into the granular component.

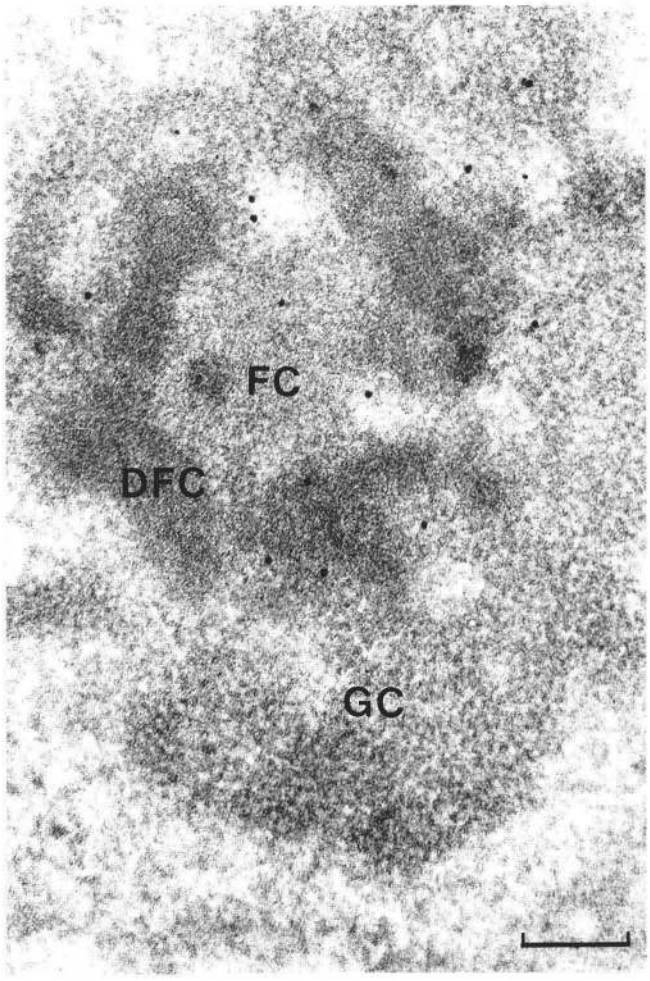
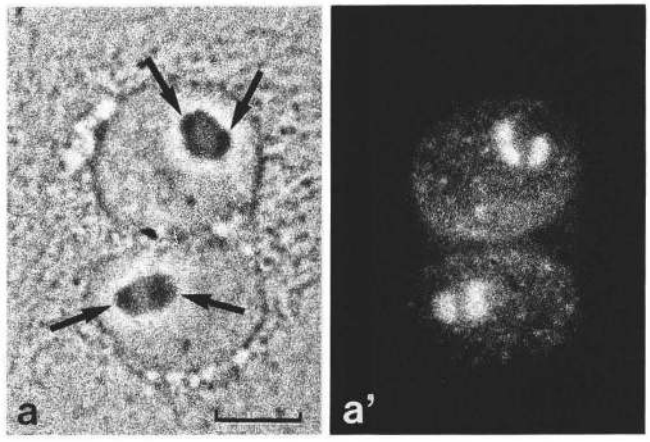


Fig. 5. Distribution of U3 RNA in the nucleolus of a cultured *Xenopus laevis* cell as revealed by in situ hybridization. *Xenopus* A6 cells were fixed with 4% formaldehyde/0.5% glutaraldehyde and embedded in Lowicryl. Ultrathin sections were hybridized with a digoxigenin-labelled riboprobe prepared by in vitro transcription of *Xenopus* U3 cDNA (kindly provided by R. Savino, R. Rivera-Leon and S.A. Gerbi) and the bound probe was visualized with monoclonal anti-digoxigenin antibodies coupled to 1 nm gold particles (Boehringer, Mannheim), followed by silver intensification with Aurion R-Gent (Biotrend, Cologne). Silver grains are recognized in the fibrillar center (*FC*), the dense fibrillar component (*DFC*) and the granular component (*GC*). Bar represents 0.5 μm

Intranucleolar localization of U3 snRNA

U3 RNA is an abundant nucleolar snRNA species that occurs in the form of RNP particles containing several proteins, including the evolutionarily highly conserved protein fibrillarin (Parker and Steitz 1987). By using mouse cell extract it has been shown that the first processing cleavage of the mammalian primary rRNA transcript near its 5′ end requires U3 snRNP (Kass et al. 1990). In contrast to these results are the in vivo findings that disruption of U3 snRNA by injection of anti-sense oligonucleotides into *Xenopus* oocytes interferes with a later processing step, i.e., correct cleavage at the ITS1-5.8S boundary (Savino and Gerbi 1990). Accordingly,



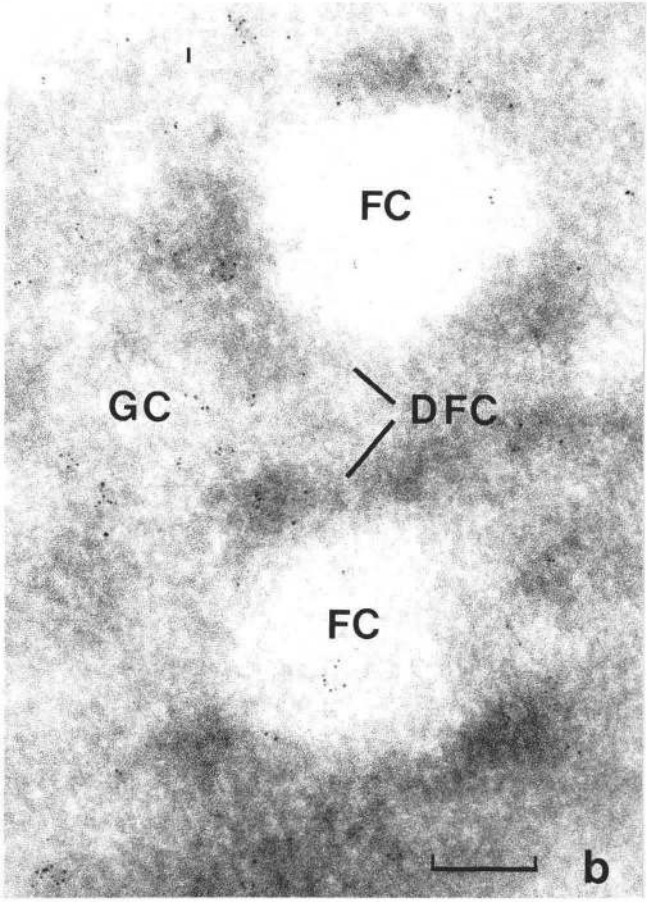


Fig. 6a, b. Localization of snRNAs by immunofluorescence (**a'**) and immunogold electron microscopy (**b**) using affinity purified rabbit antibodies against their 5'-m_3G cap structure (kindly provided by R. Lührmann; for details see Lührmann et al. 1982). The nucleolar labelling mainly reflects the distribution of U3. Nucleoli of cultured *Xenopus* A6 cells occasionally segregate spontaneously into phase-light (corresponding to the dense fibrillar component) and phase-dark (corresponding to the granular component) structures (**a**). The granular component, which appears dark in phase-contrast optics (*arrows* in **a**), is strongly fluorescing (**a'**). By electron microscopic immunogold labelling of ultrathin frozen sections of cultured rat PC12 cells (for details see Raska et al. 1989), antibodies are found in all three components of the nucleolus (**b**). The antibodies were revealed by secondary antibodies coupled to 5 nm gold particles. Bars represent 10 μm (**a**) and 0.2 μm (**b**)

U3 RNA should be localized either in the fibrillar center and/or dense fibrillar component (assuming involvement in the primary processing step) or in the granular component where later processing events occur.

In order to clarify the intranucleolar distribution of U3 RNA, digoxigenin-labelled riboprobes prepared by in vitro transcription of *Xenopus* U3 cDNA (kindly provided by R. Savino, R. Rivera-Leon and S.A. Gerbi) were hybridized in situ to Lowicryl sections of cultured *Xenopus* cells. Quite unexpectedly, U3 RNA is detected in all three nucleolar components, with some preference for the dense fibrillar component (Fig. 5). Since the granular component constitutes the bulk of the nucleolus, it apparently harbors a substantial fraction of the total nucleolar U3 RNA molecules. Further support for this conclusion comes from immunolocalization studies using antibodies directed against the 2,2,7-trimethylguanosine (m_3G) cap of the snRNAs (for a characterization of these antibodies, see Lührmann et al. 1982). Since all snRNA species except U6 possess a cap structure and most are of nucleoplasmic origin (reviewed by Reddy and Busch 1988), the anti-m_3G antibodies stain the nucleoli as well as the nucleoplasm of *Xenopus* cells in immunofluorescence preparations (Fig. 6a). The strong labelling of the nucleolus largely reflects the distribution of U3, which is the most abundant small nucleolar RNA (Tyc and Steitz 1989). Occasionally, nucleoli of cultured *Xenopus* cells undergo a spontaneous segregation (i.e., not induced by drugs such as actinomycin D), thus allowing the granular and dense fibrillar component to be identified, at the light microscopic level, as phase-dark and phase-light structures, respectively (Fig. 6a; for details, see Hügle et al. 1985a; Reimer et al. 1987). Under such conditions the granular component exhibits strong fluorescence, indicating that the majority of the U3 RNA molecules is confined to this nucleolar compartment (Fig. 6a). In contrast, antibodies to fibrillarin stain preferentially the phase-light, i.e., the dense fibrillar component (not shown; see Reimer et al. 1987). In mammalian nucleoli a sizable fraction of the U3 RNA molecules is found as well in the granular component, as shown by immunogold electron microscopy (Fig. 6b). In addition, the fibrillar centers and the surrounding dense fibrillar component are also labelled (for similar results obtained with HeLa cells, see Raska et al. 1989).

Together, these data demonstrate that the intranucleolar distribution of U3 RNA is different from that of fibrillarin. The protein fibrillarin has been detected exclusively in the fibrillar center and in the dense fibrillar component (hence the name fibrillarin; Ochs et al. 1985). Thus, fibrillarin appears to be associated only with those U3 RNA molecules that are confined to the fibrillar portion of the nucleolus. This might indicate that U3 RNA sheds its association with fibrillarin before its transit to the granular component of the nucleolus. Indeed, it has been concluded from immunoprecipitation studies that fibrillarin is associated with only a minor fraction (10 to 20%) of total cellular U3 (Parker and Steitz 1987).

Consistent with the cytological distribution of the U3 RNAs is the view that they are involved both in early and late steps of rRNA processing. U3 snRNP seems

to become an integral component of the mammalian primary rRNA processing complex near the 5′ end of the pre-rRNA (Kass et al. 1990; Kass and Sollner-Webb 1990; see also Maser and Calvet 1989; Stroke and Weiner 1989), which might be the structural counterpart to the terminal knobs of the nascent transcript fibrils visible in electron microscopic spreads of actively transcribing rRNA genes (Fig. 2a). This notion is also supported by the specific labelling of the terminal knobs with antibodies against fibrillarin (Scheer and Benavente 1990). During the subsequent processing steps U3 snRNP remains attached to the RNA products (Kass et al. 1990) and is not released until the almost mature 60S preribosomal particles leave the nucleolus (Epstein et al. 1984).

Future prospects

Our understanding of structural-functional relationships of the nucleolus will improve by the combined application of two powerful methods, viz. in situ hybridization to locate nucleic acids and immunocytochemistry to locate proteins. By using more refined and selective hybridization probes, the correlation of each step of the pre-rRNA maturation pathway with structural domains of the nucleolus is now within experimental reach. For instance, it will be interesting to determine the nucleolar location of the first cleavages of the primary transcript of mouse cells (47S pre-rRNA) that occur near its 5′ and 3′ ends and the processing events yielding both ends of the mature 18S rRNA (for refs. see Gerbi et al. 1990; Sollner-Webb and Mougey 1991). Furthermore, it should now prove possible to identify the nucleolar site of integration of 5S rRNA into the preribosome to learn more about the structural correlate of preribosomal particles en route from the nucleolar cortex to the nuclear pore complexes, and to analyze the cytological localization of the final processing events.

An intriguing feature of the nucleolus is its cyclic disintegration and disappearance during late prophase and reformation during telophase. The tools are now available to analyze the topographical distribution of the various nucleolar constituents during mitosis and their mode and sequence of interaction with each other and the chromosomal nucleolus organizer region (NOR) during the process of nucleologenesis (for details see Benavente 1991). In situ hybridization now offers the possibility of understanding in far greater detail the fate of the pre-rRNA molecules which are released from disintegrating nucleoli and how they persist during mitosis (Fan and Penman 1971) and repopulate the postmitotic nucleolus (Phillips 1972; Phillips and Phillips 1973; Hügle et al. 1985a).

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References

Benavente R (1991) Postmitotic nuclear reorganization events analyzed in living cells. Chromosoma 100:215–220

Benavente R, Rose KM, Reimer G, Hügle-Dörr B, Scheer U (1987) Inhibition of nucleolar reformation after microinjection of antibodies to RNA polymerase I into mitotic cells. J Cell Biol 105:1483–1491

Biggiogera M, Fakan S, Kaufmann SH, Black A, Shaper JH, Busch H (1989) Simultaneous immunoelectron microscopic visualization of protein B23 and C23 distribution in the HeLa cell nucleolus. J Histochem Cytochem 37:1371–1374

Das NK, Micou-Eastwood J, Ramamurthy G, Alfert M (1970) Sites of synthesis and processing of ribosomal RNA precursors within the nucleolus of *Urechis caupo* eggs. Proc Natl Acad Sci USA 67:968–975

Derenzini M, Thiry M, Goessens G (1990) Ultrastructural cytochemistry of the mammalian nucleolus. J Histochem Cytochem 38:1237–1256

Epstein P, Reddy R, Busch H (1984) Multiple states of U3 RNA in Novikoff hepatoma cell nucleoli. Biochemistry 23:5421–5425

Fakan S, Puvion E (1980) The ultrastructural visualization of nucleolar and extranucleolar RNA synthesis and distribution. Int Rev Cytol 65:255–299

Fan H, Penman S (1971) Regulation of synthesis and processing of nucleolar components in metaphase-arrested cells. J Mol Biol 59:27–42

Gerbi SA, Savino R, Stebbins-Boaz B, Jeppesen C, Rivera-Leon R (1990) A role for U3 small nuclear ribonucleoprotein in the nucleolus? In: Hill W, Dahlberg A, Garrett R, Moore P, Schlessinger D, Warner J (eds) The ribosome. ASM Press, Washington DC, pp 452–469

Goessens G (1984) Nucleolar structure. Int Rev Cytol 87:107–158

Hadjiolov AA (1985) The nucleolus and ribosome biogenesis. Springer, Berlin Heidelberg New York

Hernandez-Verdun D (1991) The nucleolus today. J Cell Sci 99:465–471

Hügle B, Hazan R, Scheer U, Franke WW (1985a) Localization of ribosomal protein S1 in the granular component of the interphase nucleolus and its distribution during mitosis. J Cell Biol 100:873–886

Hügle B, Scheer U, Franke WW (1985b) Ribocharin: a nuclear M_r 40000 protein specific to precursor particles of the large ribosomal subunit. Cell 41:615–627

Jordan EG (1991) Interpreting nucleolar structure: where are the transcribing genes? J Cell Sci 98:437–442

Karpen GH, Schaefer JE, Laird CD (1988) A *Drosophila* rRNA gene located in euchromatin is active in transcription and nucleolus formation. Genes Dev 2:1745–1763

Kass S, Sollner-Webb B (1990) The first pre-rRNA-processing event occurs in a large complex: analysis by gel retardation, sedimentation, and UV cross-linking. Mol Cell Biol 10:4920–4931

Kass S, Tyc K, Steitz JA, Sollner-Webb B (1990) The U3 small nucleolar ribonucleoprotein functions in the first step of preribosomal RNA processing. Cell 60:897–908

Lührmann R, Appel B, Bringmann P, Rinke J, Reuter R, Rothe S (1982) Isolation and characterization of rabbit anti-m_3G antibodies. Nucleic Acids Res 10:7103–7113

Maser RL, Calvet JP (1989) U3 small nuclear RNA can be psoralen cross-linked in vivo to the 5′-external transcribed spacer of preribosomal RNA. Proc Natl Acad Sci USA 86:6523–6527

Ochs RL, Lischwe MA, Spohn WH, Busch H (1985) Fibrillarin: a new protein of the nucleolus identified by autoimmune sera. Biol Cell 54:123–134

Parker KA, Steitz JA (1987) Structural analyses of human U3 ribonucleoprotein particle reveal a conserved sequence available for base pairing with pre-rRNA. Mol Cell Biol 7:2899–2913

Phillips SG (1972) Repopulation of the postmitotic nucleolus by preformed RNA. J Cell Biol 53:611–623

Phillips DM, Phillips SG (1973) Repopulation of postmitotic nucleoli by preformed RNA. II. Ultrastructure. J Cell Biol 58:54–63

Puvion E, Moyne G (1981) In situ localization of RNA structures. In: Busch H (ed) The nucleolus, vol 8. Academic Press, New York, pp 59–115

Puvion-Dutilleul F, Bachellerie JP, Puvion E (1991) Nucleolar organization of HeLa cells as studied by in situ hybridization. Chromosoma 100:395–409

Raska I, Reimer G, Jarnik M, Kostrouch Z, Raska K (1989) Does the synthesis of ribosomal RNA take place within nucleolar fibrillar centers or dense fibrillar components? Biol Cell 65:79–82

Reddy R, Busch H (1988) Small nuclear RNAs: RNA sequence, structure, and modifications. In: Birnstiel ML (ed) Structure and function of major and minor small nuclear ribonucleoprotein particles. Springer, Berlin Heidelberg New York, pp 1–37

Reeder RH (1990) rRNA synthesis in the nucleolus. TIG 6:390–395

Reimer G, Raska R, Tan EM, Scheer U (1987) Human autoantibodies: probes for nucleolus structure and function. Virchows Arch [B] 54:131–143

Savino R, Gerbi SA (1990) In vivo disruption of *Xenopus* U3 snRNA affects ribosomal RNA processing. EMBO J 9:2299–2308

Scheer U, Benavente R (1990) Functional and dynamic aspects of the mammalian nucleolus. BioEssays 12:14–21

Schmidt-Zachmann MS, Hügle-Dörr B, Franke WW (1987) A constitutive nucleolar protein identified as a member of the nucleoplasmin family. EMBO J 6:1881–1890

Sollner-Webb B, Mougey EB (1991) News from the nucleolus: rRNA gene expression. TIBS 16:58–62

Sommerville J (1986) Nucleolar structure and ribosome biogenesis. TIBS 11:438–442

Stroke IL, Weiner AM (1989) The 5′ end of U3 snRNA can be crosslinked in vivo to the external transcribed spacer of rat ribosomal RNA precursors. J Mol Biol 210:497–512

Thiry M, Thiry-Blaise L (1989) In situ hybridization at the electron microscope level: an improved method for precise localization of ribosomal DNA and RNA. Eur J Cell Biol 50:235–243

Thiry M, Scheer U, Goessens G (1991) Localization of nucleolar chromatin by immunocytochemistry and in situ hybridization at the electron microscopic level. Electron Microsc Rev 4:85–110

Tyc K, Steitz JA (1989) U3, U8 and U13 comprise a new class of mammalian snRNPs localized in the cell nucleolus. EMBO J 8:3113–3119

Warner JR (1990) The nucleolus and ribosome formation. Curr Opin Cell Biol 2:521–527